

**Amendments to the Claims:**

Please replace the abstract with following paragraph:

This invention provides isolated polynucleotides that encode the MurF (UDP-N-acetylmuramyl-L-alanine-D-glutamate-m-Dap:D-alanine-D-alanine ligase) protein of *Pseudomonas aeruginosa*. Purified and isolated MurF recombinant proteins are also provided. Nucleic acid sequences which encode functionally active MurF proteins are described. Assays for the identification of modulators of the expression of *murF* and inhibitors of the activity of MurF, are also provided.

Please replace the paragraph from line 2 to line 6 of page 6 with following paragraph:

FIGS. 1A & ~~1B~~ 1C. Nucleotide sequence (SEQ ID NO: 1) and the predicted amino acid sequence (SEQ ID NO:2) of *P. aeruginosa murF*. The amino acid sequence (SEQ ID NO:2) is presented in three-letter code below the nucleotide sequence (nucleotides 57 to 1431 of SEQ ID NO: 1).

Please replace the paragraph from line 25 to line 32 of page 6 with following paragraph:

The *murF* gene was cloned from *Pseudomonas aeruginosa*. Sequence analysis of the *P. aeruginosa murF* gene revealed an open reading frame of [[459]] 458 amino acids. The deduced amino acid sequence of *P. aeruginosa* MurF is homologous to MurF from *Escherichia coli*, *Bacillus subtilis* and other bacteria. Recombinant MurF protein from *P. aeruginosa* was over-produced as His-tagged fusion protein in *Escherichia coli* host cells and the enzyme was purified to apparent homogeneity. The recombinant enzyme catalyzed the ATP-dependent addition of D-alanine-D-alanine to the UDP-N-acetylmuramyl-L-alanine-D-Glutamine-m-Dap precursor.

Please replace the paragraph from line 24 of page 14 to line 12 of page 15 with following paragraph:

Polynucleotide probes comprising full length or partial sequences of SEQ ID NO: 1 can be used to determine whether a cell or sample contains *P. aeruginosa* MurF DNA or RNA. The effect of modulators that effect the transcription of the *murF* gene can be studied via the use of these probes. A preferred probe is a single stranded antisense probe having at least the full length of the coding sequence of *murF*. It is also preferred to use probes that have less than the full length sequence, and contain sequences specific for *P. aeruginosa murF* DNA or RNA. The identification of a sequence(s) for use as a specific probe is well known in the art and involves choosing a sequence(s) that is unique to the target sequence, or is specific thereto. It is preferred that polynucleotides that are probes have at least about 25 nucleotides, more preferably about 30 to 35 nucleotides. The longer probes are believed to be more specific for *P. aeruginosa murF*

gene(s) and RNAs and can be used under more stringent hybridization conditions. Longer probes can be used but can be more difficult to prepare synthetically, or can result in lower yields from a synthesis. Examples of sequences that are useful as probes or primers for *P. aeruginosa murF* gene(s) are Primer A (sense)

5'- TTTCATATGCTTGAGCCTCTTCGCCTC -3' (SEQ ID NO:3) and Primer B (antisense) 5'- TTGGATCCTTAGTGACTCTCCTCGGAG -3' (SEQ ID NO:4). These primers are nucleotides [[ ]] 1-21 (A) and the complement of nucleotides [[ ]] 1358-1376 (B) respectively, of SEQ ID NO:1. Restriction sites, underlined, for NdeI and BamHI are added to the 5' ends of the primers to allow cloning between the NdeI and BamHI sites of the expression vector pET-15b. However, one skilled in the art will recognize that these are only a few of the useful probe or primer sequences that can be derived from SEQ ID NO:1.

Please replace the paragraph from line 27 of page 20 to line 3 of page 21 with following paragraph:

Two oligonucleotide primers (GIBCO/BRL, Bethesda, MD) complementary to sequences at the 5' and the 3' ends of *P. aeruginosa murF* were used to clone this gene using KLENTAQ ADVANTAGE™ polymerase (CLONTECH, Palo Alto, CA). The primer nucleotide sequences were as follows:

5'- TTTCATATGCTTGAGCCTCTTCGCCTC -3' (SEQ ID NO:3) (a NdeI linker plus nucleotides [[ ]] 1-21 of SEQ ID NO: 1) and

5'- TTGGATCCTTAGTGACTCTCCTCGGAG -3' (SEQ ID NO:4) (a BamHI linker plus the complement of nucleotides [[ ]] 1358-1376 of SEQ ID NO: 1). A PCR product representing *P. aeruginosa murF* was verified by nucleotide sequence, digested with NdeI and BamHI, and cloned between the NdeI and BamHI sites of pET-15b, creating plasmid pPaeMurF. This plasmid was used for expression of the *murF* gene in *E. coli*.

Please delete the paragraph beginning at page 21, line 4, which starts with "The plasmid pPaeMurF".

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claim 1 (currently amended): A purified and isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having ~~[[an]]~~ the amino acid sequence of SEQ ID NO: 2, and

(b) a polynucleotide which is complementary to the polynucleotide of (a), ~~and~~

~~————— (c) ——— a polynucleotide that hybridizes with a polynucleotide of (a) or (b) under stringent conditions.~~

Claim 2 (previously presented): The polynucleotide of claim 1 wherein the polynucleotide comprises nucleotides selected from the group consisting of natural, non-natural and modified nucleotides.

Claim 3 (previously presented): The polynucleotide of claim 1 wherein the internucleotide linkages are selected from the group consisting of natural and non-natural linkages.

Claim 4 (previously presented): The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1.

Claim 5 (currently amended): ~~A polynucleotide that is an~~ An expression vector comprising a polynucleotide of claim 1.

Claim 6 (previously presented): A host cell comprising the expression vector of claim 5.

Claim 7 (currently amended): A process for expressing a MurF protein of *Pseudomonas aeruginosa* in a recombinant host cell, comprising:

- (a) transforming a suitable host cell with an expression vector of claim 5; and  
[[,]]
- (b) culturing the host cell of step (a) in conditions under which allow expression of said the MurF protein from said expression vector.

Claim 8 (currently amended): A purified and isolated polypeptide having [[an]] the amino acid sequence of SEQ ID NO:2.

Claim 9 (previously presented): A method of determining whether a candidate compound is an inhibitor of a *Pseudomonas aeruginosa* MurF polypeptide comprising:

- (a) providing at least one host cell harboring an expression vector that includes a polynucleotide encoding a polypeptide having an amino acid sequence of SEQ ID NO: 2,
- (b) contacting at least one of said cells with the candidate to permit the interaction of the candidate with the MurF polypeptide, and
- (c) determining whether the candidate is an inhibitor of the MurF polypeptide by ascertaining the relative activity of the polypeptide in the presence of the candidate.

Claim 10 (previously presented): The method of claim 9 wherein the polynucleotide has the nucleotide sequence of SEQ ID NO:1.

Claim 11 (previously presented): The method of claim 9 wherein in step (c) the relative activity is determined by comparing a measurement of MurF polypeptide activity of at least one cell before step (b) to a measurement of MurF polypeptide activity of at least one cell after step (b).

Claims 12-14 (canceled)

Claim 15 (previously presented): A method of determining whether a candidate compound is an inhibitor of a *Pseudomonas aeruginosa* MurF polypeptide comprising:

- (a) providing a sample that includes a MurF polypeptide having an amino acid sequence of SEQ ID NO: 2,
- (b) contacting said sample with the candidate to permit the interaction of the candidate with the MurF polypeptide, and
- (c) determining whether the candidate is an inhibitor of the MurF polypeptide by ascertaining the relative activity of the MurF polypeptide in the presence of the candidate.

Claim 16 (previously presented): The method of claim 15 wherein the polypeptide has the amino acid sequence of SEQ ID NO:2.

Claim 17 (previously presented): The method of claim 15 wherein in step (c) the relative activity is determined by comparing a measurement of MurF polypeptide activity of the sample before step (b) to a measurement of MurF polypeptide activity of the sample after step (b).